1. (Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

(i) incubating the nucleic acid sample with a primer, DNA polymerase, and a deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or a dideoxynucleotide triphosphate;

(ii) measuring the pyrophosphate released in step (i); and

(iii) identifying the nature of the nucleotide base added by measuring which nucleotide caused the release of pyrophosphate in step (ii)

wherein steps (i) to (iii) are performed in a microfluidic device.

2. (Amended) A method for identifying the sequence of a portion of sample DNA comprising the steps of:

(i) forming immobilised double stranded DNA on one or more reaction areas in a microchannel structure of a microfluidic device;

(ii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase to each of said one or more reaction areas so that extension of primer only occurs if there is a complementarity of the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the strand of sample DNA that is part of the immobilised double stranded DNA;

(iii) detecting whether or not the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added in step (ii) has been added to the primer DNA in said one or more reaction areas; and

(iv) repeating steps (ii) and (iii) as required with a different deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide.

3. (Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

(i) attaching 0.1 – 200 pmol of a primer or single stranded DNA sample to each of between one and 100,000 pre-determined areas on the surface of a microfluidic device;

(ii) hybridising small amounts of single stranded sample DNA or primer respectively to each of the predetermined areas;

(iii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase so that extension of the primer only occurs, with consequent release of pyrophosphate, if there is a complementarity with the sample DNA;

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(iv) measuring the release of pyrophosphate and from which predetermined area on the device it is released; and

(v) repeating steps (iii) and (iv) as required to construct a DNA sequence for the elongated primers, and hence for portions of the sample DNA.

4. (Amended) A method for identifying the sequence of a portion of sample DNA, comprising the steps of:

(i) adding sample DNA to a predetermined area on a microfluidic device;

(ii) moving the sample to a reaction chamber on the microfluidic device;

(iii) attaching the sample DNA to a surface of the reaction chamber, wherein a primer is hybridised to the DNA;

(iv) extending the primer in the presence of a DNA polymerase with a deoxynucleotide, deoxynucleotide analogue, or dideoxynucleotide, wherein the extension is indicated by detection of pyrophsophate released from the extension reaction; and

(vi) repeating step (iv) as required to establish the sequence of the extended primer.

5. (Amended) The method of claim 1, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

6. (Amended) The method of claim 2 wherein the detection step involves labeled terminator.

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7. (Amended) The method claim 1, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

8. (Amended) The method of claim 1, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

Please add the following new claims.

9. The method of claim 3, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

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10. The method of claim 4, wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

11. The method claim 2, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

12. The method of claim 2, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

13. The method claim 3, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide incorporation is performed in real time.

14. The method of claim 3, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

15. The method claim 4, wherein the detection of the deoxynucleotide, deoxynucleotide analogue, dideoxynucleotide, or dideoxynucleotide analogue incorporation is performed in real time.

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16. The method of claim 4, wherein the microfluidic device is a disc and the fluids are moved by centripetal force.

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REMARKS/ARGUMENTS

Claims 1-8 were in the original PCT application as filed. Applicants have amended claims 1-8 to delete the multiple dependency. Applicants have also added new claims 9-16, which relates to the subject matter that was contained in the multiple dependent claims of the PCT application. Applicants have included a marked up version of the claims as amended herein as Appendix A. For the convenience of the Examiner, Applicants have included in Appendix B a copy of all pending claims as amended herein. Applicants assert that no new matter has been added.

CONCLUSION

Claims 1-8 were in the original PCT application. Applicants have amended claims 1-8 to delete the multiple dependency and have added new claims 9-16 which related to the subject matter in the original multiple dependent claims. Therefore, these amendments do not narrow the scope of the claims within the meaning of *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.*, 234 F.3d 558, 586, 56 USPQ2d 1865, 1886 (Fed. Cir. 2000).

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

By

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